Conditioning was TBI-based in 80/81 pts and GVHD prophylaxis was with Cyclosporine and short-course Methotrexate (MTX) (15 mg/m^2 d + 1 and 10 mg/m^2 d + 3, d + 6 and d + 11). Fifty of 81 pts received >80% of the planned MTX dose. Forty three pts received SCT from a 10/10 high-resolution HLA-matched donor, 25 pts received a 1-antigen mismatched SCT, 12 pts received a 2-antigen mismatched SCT and 1 pt a 3-antigen mismatched SCT. The donor was male for 68 pts and female for 13 pts. Median CD 34 cell dose was 7.75 $\times$ 10^6 (range-9.46 $\times$ 10^4–33.6 $\times$ 10^6)/kg. **Results:** The estimated 3-year NRM, RR and OAS were 39% (95%CI 25%-50%), 30% (95%CI 17%-41%) and 43% (95%CI 31%-58%), respectively. In multivariate analysis, CD 34 cell dose >7.75 $\times$ 10^6/kg was associated with faster neutrophil engraftment, $p < 0.001$ and reduced NRM (28% vs. 49%, $p = .019$), but did not influence the incidence of either acute or chronic GVHD or OAS. On multivariate analysis the most important predictor of reduced grade 3–4 acute GVHD (22% vs. 49%, $p = .005$), NRM (30 vs. 65%, $p = .006$), and improved OAS (50% vs. 24%%) was administration of >80% of the planned MTX dose. **Conclusion:** A higher CD34 cell dose in unrelated donor SCT does not adversely influence the incidence of GVHD and is associated with faster neutrophil engraftment and a reduction in NRM. Delivery of at least 80% of the planned short-course MTX GVHD prophylaxis continues to be critical in producing a favourable outcome.

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THE RELATION BETWEEN THE FREQUENCY OF PRESENCE OF THROMBOPHILIA AND THROMBOEMBOLIC COMPLICATIONS AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION (AHCT)

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Venous thromboembolism (TE) occurs as a consequence of genetic and environmental factors. Important genetic risk factors are deficiencies of natural anticoagulants, antithrombin-III (AT-III), protein C (PC) and PS, and genetic mutations of factor V leiden (FVL) and prothrombin (PTH A20210). Thrombotic complications after HCT are usually catheter-related thrombosis (CRT), pulmonary TE (PTE) and deep vein thrombosis (DVT). Our aim in this study was to evaluate the effects of the deficiencies of ATIII, PC and PS, and the gene mutations of FVL and PTH on the incidence of the development of TE complications and liver sinusoidal obstruction syndrome (SOS) at the early or late period post-HCT. In our center, pre-transplant work-up includes routine thrombophilia tests. Between Apr 1999-Jan 2007 260 patients (M/F: 145/115, median age: 34 years) admitted to our transplantation center were retrospectively analyzed for the relation of the presence of thrombophilia and the frequencies of the occurrence of a TE complications and liver SOS. All but 6 patients (n = 254) underwent allogeneic HCT from an HLA identical sibling donor. The ratios of the detection of the plasma activation level below 50% for PC, PS and AT-III were 5.8% (12/206), 15.7% (32/204) and 25.4% (66/260), respectively. Gene mutations were studied in 198 patients prior to transplant and the frequencies of gene mutations were detected in 11.6% patients (n = 23) either FVL (n = 14) or PTH (n = 9) gene. None of the patients had both mutations, FVL and PTH. At the peri- or post-transplantation period we observed venous TE in 24 patients (17 CRT, 2 PE and 5 DVT), liver SOS in 23 patients and myocard infarction at the early period in only one patient. In 4 out of 12 patients with low PC activity CRT occurred, and liver SOS was observed in only one patient. In 6 out of 24 patients with genetic mutation developed a TE complication, 5 CRT and 1 PTE. CRT occurred in 4 of 14 patients with FVL mutation, while 1 CRT and 1 PTE among 9 patients with PTH mutation were observed. Liver SOS was seen in only one patient with PTH heterozygote positive. We found the presence of low PC level or genetic mutation increased the frequency of the development of TE (OR: 6.9 and 3.7, respectively), but no effect on the liver SOS. In conclusion, the use of thromboprophylaxis at peri-transplant period in patients with genetic mutations is still a controversial topic; which should be elucidated with controlled studies.

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ONCE DAILY BUSULFAN CYCLOPHOSPHAMIDE IS WELL TOLERATED AND EFFECTIVE PRIOR TO ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Intravenous busulfan (Bu) allowes for the delivery of Bu in one dose instead of dividing 4 times per day. Pharmacokinetic (PK) studies of Bu support the feasibility of once daily dosing, and prior clinical studies found no unexpected toxicity of once daily Bu when combined with fludarabine. Based on this data we began an institutional protocol of delivering Bu once daily follwed by standard cyclophosphamide (CY) and allogeneic hematopoietic stem cell transplantation (HCT). We report a retrospective review of our institutional data using once daily BuCY vs 4× daily BuCY and total body irradiation (TBI)/CY in pts who received allogeneic HCT from January 2000 to December 2006. Bu 3.2 mg/kg daily × 4 days followed by CY 60 mg/kg daily × 2 days was given to 42 patients (pts). Bu 0.8 mg/kg given 4× daily for 4 days followed by CY 60 mg/kg was given to 15 pts. CY 60 mg/kg daily for 2 days and fractionated TBI 1200 cGy delivered over 3 days was given to 60 pts. All donors were HLA matched at A, B, C, DR, and DQ and were related/unrelated in 23/19, 11/4, and 21/39 in the once daily BuCY, 4× daily BuCY, and TBI/CY, respectively. Significantly more pts with myeloid leukemias received a BuCY regimen and significantly more pts with lymphoid malignancies received TBI/CY. Median follow up for all pts was 370 days. VOD developed in 2 pts in the once daily BuCY group, 1 pt in the 4× daily BuCY group, and in no pts in the TBI/CY group. Acute GVHD grade II-IV occured in 33% of the once daily BuCY pts, 53% of the 4× daily BuCY pts, and 32% of the TBI/CY pts. Estimated actuarial transplant related mortality (TRM) and survival are described in the table below. There was no statistical difference in TRM or survival between the once daily and 4× daily BuCY groups or the total BuCY group and TBI/CY group. The once daily BuCY group had significantly less TRM than the TBI/CY group at 100 days ($p = 0.04$) and at 1 year ($p = 0.01$). The once daily BuCY group also had significantly better survival at one year compared to the TBI/CY group ($p = 0.01$), but this became non-significant at 3 years. The significant differences in the pt populations treated in the BuCY and TBI/CY groups, as well as the retrospective nature of this study, limit the ability to draw conclusions comparing the groups. However, this review does demonstrate once daily BuCY and allogeneic HCT is well tolerated with no unexpected TRM, and provides good long term survival in pts with myeloid malignancies.

Survival and TRM

	1 × BuCY (95%CI)	4 × BuCY (95%CI)	CY/TBI (95%CI)
100 day TRM	7% (1–16)	21% (7–51)	22% (15–35)
1 year TRM	21% (11–38)	37% (17–69)	45% (33–59)
1 year Survival	70% (53–82)	47% (21–69)	49% (35–61)
3 year Survival	43% (24–59)	27% (8–50)	37% (24–49)

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THE IMPACT OF THE DISPARITIES OF SHORT TANDEM REPEATS (STR) BETWEEN DOONOR-RECIPIENT PAIR ON THE ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION (AHCT) OUTCOME

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STR marker systems are used in the monitoring of hematopoietic chimerism in patients after AHCST. The biological effect of STR disparity on AHCT outcome has rarely been studied. Therefore, we aimed to evaluate the impact of STR disparity on allo-HCT outcome in our single center. Between Sept 2001 and Feb 2007, 150 patients (81M/69F, median age: 34 ys) underwent AHCT (Stem cell

source: 119 PB/31 BM) were retrospectively analyzed. Their diagnoses were 90 acute leukemia, 30 CML, 6 MDS and 24 other diseases. Multiplex PCR method was performed to amplify 16 STR loci (D3S1358, HUMvWA, D16S539, D2S1338, Amelogenin, D8S1179, D21S11, D18S51, D19S433, THO1, FGA, D7S820, CSF1PO, D13S317, TPOX, D5S818) (ABI Prism 3130). The loci examined were classified as complete matched (CM), partially matched (PM), and fully mismatched (FMM) between donors and recipients.

Results: The loci of D13S317, D18S51 and D2S1338 were the most informative, while the loci of TPOX and CSF1PO were the least. The incidence of acute GvHD was 46.7% (n = 69), which acute severe GvHD (grII-IV) was observed in only 31 patients. Chronic GvHD was developed in 63.4% patients. The incidence of grII-IV GvHD was higher in patient with CM in TPOX loci (p = 0.02). Chronic GvHD was more frequent in the patients with PM in D5S818 loci than those with CM or MM (p = 0.016). While PM D21S11 increased TRM, MM or PM in D5S818 loci decreased the TRM. In our cohort analysis, 2-year probability of disease-free survival(DFS) and overall survival(OS) were 58.1 ± 5.5% and 67.5 ± 4.4%, respectively. The CM in D21D11 locus (p = 0.07) and PM in D5S818 locus (p = 0.009) prolonged the probability of DFS. In multivariate analysis, these loci had an impact on DFS (p = 0.055 ve p = 0.005). D19S433 and D5S818 loci had an effect on the OS. We repeated similar analyses into two groups, mismatched (MM) group, which FMM and PM was accepted as a whole group or CM group, while the incidence of grII-IV GvHD was higher in patients with CM of D18S51 and TPOX loci, the chronic GvHD was more frequent in those with CM D5S820 loci. Similar as the first analyses MM in D21S11 and CM in D5S818 affected both TRM and DFS. Besides, MM in FGA locus decreased TRM and prolonged DFS. The impact of D5S818 on the OS also continued, additionally D19S433 had minimal effect on the OS. In conclusion, some disparities of STR loci might affect the transplantation outcome; however, these results should be analyzed together with other co-variates and on multicenter basis.

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AN EVALUATION OF DNA METHYLATION CHANGES IN A PHASE I CLINICAL TRIAL OF LOW-DOSE 5-AZACITIDINE (AZA) GIVEN AS MAINTENANCE THERAPY AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)

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DNA methylation of CpG Islands is a frequently found epigenetic alteration in AML and MDS that leads to deranged gene activity. Inhibition of the enzyme DNA methyltransferase is associated with hypomethylation, and possibly, restoration of normal function to genes critical for differentiation and proliferation. AZA is a DNA hypomethylating agent that may induce increased tumor immunogenicity, potentially magnifying the GVL effect. Lower doses are likely to be better tolerated after HSCT and to be effective inducers of hypomethylation. We hypothesized that AZA after HSCT will lower relapse rates, and designed a phase I clinical trial that also uses a molecular surrogate endpoint for dose finding. Here we describe methylation changes in patients so treated.

Pts with AML or high-risk MDS not in first remission (CR) are eligible. Three doses of AZA were studied: 8, 16, and 24 mg/m^2 daily × 5 starting on day + 42. DNA was extracted from mononuclear cells of 22 pts that received AZA. The methylation status of long interspersed nuclear elements (LINE) was analyzed by pyrosequencing as a surrogate marker of global DNA methylation before and after AZA administration. Gene specific methylation changes were studied using a methylated CpG island amplification(MCA)/CpG array.

Median age was 57 years. Diagnoses were MDS (n = 4) and AML(n = 18). Disease status at HSCT: CR, 18% (n = 4), and active disease, 82%(n = 18). LINE methylation results were as follows: baseline: 44.51%; on cycle 1, 5th day of AZA: 24 mg/m^2 = 43.58%; 16 mg/m^2: 36.93%; 8 mg/m^2: 42.86%. Two pts have relapsed while on AZA. There has been no major drug-related toxicity and no increase in GVHD incidence. Analysis of gene-specific methylation is ongoing.

AZA in doses up to 24 mg/m^2 can be safely administered early after HSCT. Given the lack of toxicity and low levels of changes in DNA methylation observed, we are currently investigating higher doses.

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ROLE OF EXTENSIVE SPLENOMEGALY IN PATIENTS WITH MYELOFIBROSIS UNDERGOING A REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANT

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Allogeneic stem cell transplantation has been shown to restore normal hematopoiesis in patients with intermediate or high risk primary myelofibrosis (PMF) or myelofibrosis preceeded by polycythemia vera or essential thrombocythemia. However, in patients with PMF and extensive splenomegaly it is not clear whether transplant is associated with an unacceptable risk or if splenectomy should be performed prior to transplant. In this study, ten consecutive patients with myelofibrosis who were not splenectomized received an allogeneic hematopoietic stem cell transplantation (HSCT) from related or unrelated donors and were periodically monitored by ultrasound or CT scan for 12 months after transplant to assess the kinetics of the reduction of splenomegaly. These findings were correlated with the time to resolution of marrow fibrosis, time to engraftment and clinical outcome. Over a 12 month period a progressive reduction in spleen size was observed in all the patients and paralleled the reduction in marrow fibrosis. Of 10 patients, 5 with a splenic longitudinal diameter >30 cm showed a more prolonged time to engraftment ANC > 0.5 × 10^9/L (d 19 ± 5 vs 13 ± 2, p = 0.05) and platelet > 20 × 10^9/L (d 75 ± 104 vs 11 ± 2, p = 0.06). However, of the 5 patients with more extensive splenomegaly only 2 experienced delayed engraftment of platelets (d 77 and 256, respectively). Full donor chimerism was observed in all patients within 60 days after transplantation regardless of the size of the spleen. At a median follow-up of 51 months (range: 2–81) all the patients are alive but 1 who died of a TTP like syndrome 2 months after transplant and had a small spleen at the time of transplant. A clinical CR has been documented in 7 patients and 2 have achieved clinical improvement according to to the criteria established by the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT). These findings suggest that allogeneic HSCT with RIC regimen can be safely performed in patients with extensive splenomegaly. Although in these patients the time to achieve a full hematopoietic engraftment may be prolonged, the significant risk of morbidity and mortality associated with splenectomy may be avoided.

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EFFECT OF SUBSTITUTING FLUDARABINE AND THYMOGLOBULIN FOR CYCLOPHOSPHAMIDE IN BUSULFAN-BASED CONDITIONING REGIMENS ON T-CELL CHIMERISM AND OUTCOMES AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION (HCT)

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Substitution of cyclophosphamide by agents such as fludarabine and thymoglobulin in busulfan-based conditioning regimens has been used to reduce complications after allogeneic HCT. We performed a retrospective analysis of outcomes among 348 patients